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Toxicology

Short-term mercury exposure on Na^+/K^+-ATPase activity and ionoregulation in gill and brain of an Indian major carp, *Cirrhinus mrigala*

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ABSTRACT

Recently mercury pollution has been increased considerably in aquatic resources throughout the world and it is a growing global concern. In this study, the 96 h LC50 value of waterborne mercuric chloride for *Cirrhinus mrigala* was found to be 0.34 mg/L (with 95% confidence limits). Fingerlings of *C. mrigala* were exposed to 0.068 and 0.034 mg/L of mercuric chloride for 96 h to assess the Na^+/K^+-ATPase activity and ionoregulation (Na^+, K^+ and Cl^-) in gill and brain. Results showed that Na^+/K^+-ATPase activity and ionic levels (Na^+, K^+ and Cl^-) in gill and brain of fish exposed to different concentrations of mercuric chloride were found to be significantly ($p < 0.05$) decreased throughout the study period. Mercury inactivates many enzymes by attaching to sulfur atoms in which the enzyme Na^+/K^+-ATPase is highly sensitive to mercury. The inhibition of gill and brain Na^+/K^+-ATPase activity might have resulted from the physicochemical alteration of the membrane due to mercury toxicity. Moreover, inhibition of Na^+/K^+-ATPase may affect the ion transport and osmoregulatory function by blocking the transport of substances across the membrane by active transport. The present study indicates that the alterations in these parameters can be used in environmental biomonitoring of mercury contamination in aquatic ecosystem.

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Introduction

Metals are naturally occurring components that are ubiquitous in the earth's crust [1,2]; unfortunately it is considered to be a major aquatic problem [3]. Aquatic contamination by heavy metals is a major ecological and health concerns worldwide [4]. Among the heavy metals, mercury (Hg) is one of the nonessential toxic heavy metal and found everywhere in the earth [5,6]. Recently mercury pollution has increased considerably and it is a growing global concern [7]. Mercury exists in the environment in several physical and chemical forms like vapor elemental Hg (Hg°), inorganic Hg (Hg^{+2}), and organic Hg (CH_3Hg) which are non-biodegradable in nature [8,9]. Mercury enters the body of living organism in the form of inorganic salts or organic or elemental mercury that has diverse toxicological profiles at the cell, organism, and ecosystem level [10]. The atmospheric mercury can be transported from one place to another place and may convert to methylmercury (CH_3Hg^+) and accumulated in the food chain causing a serious threat to human health which results in neurological disorder and death [11–15]. Asian countries become the main contributor of atmospheric mercury (Hg), accounting half of the global emission [12].

In aquatic environment, mercury is present in many physical and chemical forms with a range of properties, consequently determining complex distribution, bioavailability and toxicity patterns [16,17]. Normally, in freshwater environment, inorganic form of mercury is present in large quantities [8,18]. Due to its high toxicity and its widespread occurrence in the environment, its monitoring has attracted special attention [17]. Hence the understanding of toxicant uptake, behavior and responses in fish may perhaps, have a high ecological significance. Fish is the top most organism of the aquatic food web and are susceptible to waterborne mercury toxicity and most of the studies are assessed on central nervous system (CNS) and olfactory organs [19]. To assess the possible disturbances in the physiology of fish, suitable biomarkers are used to monitor the environmental contamination of xenobiotics.

Enzymes are sensitive biochemical marker for metal contamination in aquatic ecosystem. In aquatic organisms particularly in fish gills, the enzyme Na^+/K^+-ATPase is play a major role in the maintenance of ion balance [20] and its activity (increase or decrease) prove to be an vital index for tolerable levels of environmental contaminants and also as a potential indicators of toxic stress [21–23]. Further, Na^+/K^+-ATPase activity can be used as an early warning of pollutant, because the inhibition of this enzyme occur before gross osmoregulatory dysfunction [24]. Brain is more vulnerable for mercury poisoning, particularly for inorganic form, which disrupts Na^+/K^+-ATPase system, ionoregulatory activities and neurological

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function [19,25,26]. The changes in the tissue or an organ or group of organism are measured using various biomarkers, in which electrolytes are considered as a sensitive biomarker, because even a low concentration of waterborne metals may disrupt ionoregulation [18,27–29]. The changes in the ionoregulation activity lead to cardiovascular collapse and then ultimately death [30].

In India, approximately 200 t of mercury and its compounds are released into the environment annually as effluents from various industrial sectors [1,31]. Groundwater, fish and sediment samples from different states like Punjab, Haryana, Mumbai, Maharashtra, Tamilnadu, etc., showed surprisingly high levels of Hg [32–34]. To our knowledge study on neuro-toxicological effects of mercury in Indian major carps are very limited. Hence the present study has been carried out to assess the acute toxicity of mercuric chloride on gill and brain Na^+/K^+-ATPase activity and ionoregulation of an Indian major carp *Cirrhinus mrigala*. The carp is endemic to Indo-Gangetic riverine systems, cultivated widely in Southeast Asian countries. The carp was taken as a test species due to its commercial importance, taste and also a suitable indicator for monitoring of environmental pollution.

Materials and methods

Fish and maintenance in the laboratory

Specimens of *C. mrigala*, was selected as an experimental animal model. Fish with an average weight of 8.0 ± 0.5 g and length of 6.0 ± 0.5 cm were purchased from Aliyar Fish Farm, Aliyar, Tamilnadu and India. Fish were safely brought to the laboratory in well-packed aerated polythene bags. After arriving to laboratory, fish were stocked in a large cement tank (1000 L capacity) for a minimum period of 25 days. During acclimation period fish were fed *ad libitum* with rice bran and ground nut oil cake in dough form once in day before replacement of water. Three forth of the water was changed daily to remove excess feed and fecal materials. Dechlorinated tap water was used throughout the study period, with the following hydrological features such as; temperature 26.2 ± 1.5 °C, pH 7.1 ± 0.05, salinity 0.27 ± 0.7 ppt, dissolved oxygen 6.6 ± 0.04 mg/L and total hardness 17.1 ± 0.8 mg/L. Before the commencement of the experiment, healthy fingerlings of *C. mrigala* were transferred to clean glass aquarium tanks (200 L capacity) and that served as the stock for the experimental schedule.

Toxicity assessment of 96 h LC50 value

Preliminary toxicity tests were carried out to determine the median lethal tolerance limit of fish *C. mrigala* to mercuric chloride for 96 h. Separate circular plastic water tubs (50 L) were taken and different concentrations of mercuric chloride such as 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg/L were added. Then 10 healthy fish from the stock were randomly collected and introduced into each tub, which were starved for a period of 48 h prior to the experiment. To each concentration three replicates were maintained. Simultaneously, a control group (toxicant free) was also maintained in three different aquaria under identical conditions. The mortality/survival of fish in control and mercuric chloride tubs were recorded after 96 h. The median lethal concentration for 96 h was found to be 0.34 mg/L, which was calculated by probit analysis method of Finney [35] and homogenicity of the population was tested using chi-square test of Busvine [36]. The dead fish in the tank were removed immediately.

Short term toxicity studies

For acute toxicity study, six tubs with 50 L of capacity were taken and divided into two groups with three tubs in each. One group of tubs were received 1/5th (0.068 mg/L) value of 96 h LC50 of mercuric chloride (Treatment I) and the other group were received 1/10th (0.034 mg/L) value of 96 h LC50 of mercuric chloride (Treatment II). To each tub 15 fish from the stock were introduced. A control was also maintained with similar setup. After 96 h, fish from the Control, Treatment I and Treatment II were randomly collected and organs (gill and brain) were removed for the estimation of Na^+/K^+-ATPase activity and ionoregulation (Na^+, K^+ and Cl^-).

Sample preparation

Fish were thoroughly washed with double distilled water and dehydrated with absorbent paper. 100 mg of gill and brain were removed from the Control, Treatment I and Treatment II groups and homogenized with 1.0 mL of 0.1 M Tris–HCl buffer (pH 7.5) in ice-cold condition. The homogenate was centrifuged at 1000 rpm for 15 min at −4 °C, then, the supernatant was used for the estimation of Na^+/K^+-ATPase activity and ionoregulation (Na^+, K^+ and Cl^-).

Assessment of Na^+/K^+-ATPase activity

To determine the Na^+/K^+-ATPase activity, 100 mg of gill and brain tissue from Control, Treatments I and II were collected and homogenized with 1.0 mL of 0.1 M Tris–HCl buffer (pH 7.4) in ice-cold condition using a Teflon homogenizer and the contents were centrifuged at 1000 rpm at 4 °C for 15 min. The supernatant was used for the estimation of Na^+/K^+-ATPase activity [37] and the values were expressed as μg/h/g.

Estimation of ionoregulation

Estimation of sodium and chloride. Sodium and chloride level in gill and brain was estimated following the method of Maruna [38]. To determine the sodium level 0.01 mL of sample from Control, Treatments I and II was taken in a test tube and to this 1.0 mL of precipitating reagent was added. To the tube marked as standard, 0.01 mL of standard reagent was added. All the test tubes were mixed well and allowed to stand at room temperature for 5 min. Then the contents were centrifuged at 2000–3000 rpm for 2 min to obtain a clear supernatant. To 0.02 mL of the supernatant 1.00 mL of color reagent was added mixed well and allowed to stand at room temperature for 5 min. A standard tube was also used with 0.01 mL of standard reagent. The optical density of the Control, Standard, Treatments I and II were measured against distilled water using UV Spectrophotometer at 530 nm within 10 min and readings were expressed as mmol/L.

To determine the chloride level, 10 mL of supernatant from Control, Treatments I and II were taken in a test tube and to this 1000 μL of thiocyanate reagent was added. Similarly, 10 mL of distilled water was taken in a test tube and marked as blank. For standard, 10 mL of standard chloride reagent was added to the tube marked as standard. Then the contents of the tubes were mixed well and kept for 10 min at room temperature and the optical density of Control, Standard, Treatments I and II were measured against 'Blank' using UV Spectrophotometer at 505 nm and the readings were expressed as mmol/L.

Estimation of potassium. Potassium level in gill and brain were estimated the following the method of Young et al. [39] and Tietz [40]. 1.0 mL of boron reagent was taken in a test rube and to this 0.05 mL of supernatant from Control, Standard, Treatments I and II were added. To the test tube marked as standard 0.05 mL of potassium standard was added. Then all the tubes were mixed well and allowed to stand for 10 min at room temperature. After 10 min the absorbance of samples were measured against distilled water using UV Spectrophotometer at 620 nm and the readings were expressed as mmol/L.

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